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La Dolce Vita: A Molecular Feast in Plant–Pathogen Interactions

Meeting Review

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Plants are sessile organisms and lack a circulating, somatically adaptive immune system to protect themselves against pathogens. They instead have evolved other mechanisms for defense against a spectrum of pathogens. Plants are, in fact, resistant to most microorganisms by means of constitutive chemical or physical barriers such as cuticular coats of wax armor that a potential pathogen must penetrate or bypass. Disease is therefore the exception rather than the rule when microbes and plants meet. Yet yield loss due to plant disease remains an important component of modern agriculture, as many pathogens are evolutionarily specialized to overcome preformed defense barriers. Plant defense is based on recognition of specific pathogen molecules and subsequent induction of a broad defense response. Recognition evolves germinally, so that an individual plant can only defend itself with the spectrum genes it inherited from its parents. Genetic diversity among individuals is therefore essential for survival of the host species against rapidly evolving pathogens.

Specific pathogen recognition is governed genetically by interactions between the product of a disease resistance (*R*) gene in the host and molecules encoded in a given pathogen isolate by so-called avirulence (*avr*) genes. If either the host plant or pathogen isolate lack the corresponding *R* or *avr* allele, then the pathogen can continue to colonize the host, reproduce, and ultimately cause disease. Alternatively, matching *R* and *avr* alleles enable pathogen recognition and a subsequent series of intracellular signal events in the host (see below). The simplest mechanistic interpretation of the genetics would posit *R* products as receptors for *avr*-encoded ligands. Recognition typically leads to rapid localized cell death of host cells penetrated by the pathogen, termed the hypersensitive response (HR). In many plant species, the local HR initiates a systemic response by which distal tissues in the host become resistant to secondary infection. In contrast to specific memory in mammalian immune systems, plant systemic acquired resistance (SAR) is pathogen nonspecific. Thus, for example, the response of a lower leaf to attempted infection by a bacterial pathogen can lead to protection against subsequent infection by, for example, a fungal pathogen.

A detailed understanding of pathogen recognition signaling of the defense response will contribute to engineering of disease resistance in crops. Disease resistance mediated by genotype-specific pathogen recognition has recently emerged as a major topic for investigating signal transduction in plants, catalyzed by the cloning of at least 10 functional *R* genes from Arabidopsis, tomato, tobacco, flax, and rice (reviewed by Dangl, 1995; Bent, 1996; Hammond-Kosack and Jones, 1996b; Baker et al., 1997). These confer resistance to bacterial, viral, fungal, and nematode pathogens with very different extracellular and intracellular lifestyles. Thus, it was astounding that all of these *R* genes encode a limited set of products with related structural features. The cloning of *R* genes continues apace and has recently been augmented by isolation of other key signal transduction components. Additionally, the last year has produced evidence that bacterial plant pathogens probably "inject" virulence and avirulence proteins directly into eukaryotic host cells. This is consistent with the requirement for evolutionarily conserved Type III secretion systems in these pathogens and with the finding that bacterial pathogens of mammals also use this system to deliver virulence factors to host cells.

Current key questions in this research field include: what are the structures of plant R proteins? How is specificity determined, how do new specificities evolve, and how is genetic diversity organized and maintained in the host species? Do all R proteins directly interact with pathogen avr proteins? What are the molecular steps required for R-dependent recognition to be translated into disease resistance as measured by killing or inhibiting pathogen growth? What are the positive functions of avr proteins for pathogens? Recent advances to answer these questions were addressed by nearly 80 participants in a recent sun-bathed EMBO Workshop in Maratea, Italy organized by Jonathan Jones and Giulia DeLorenzo.

The Structural Variety of R Proteins

Leucine-Rich Repeats as Specificity Determinants

The most striking feature in all known R proteins, with one exception described below, is a variable number of Leucine-Rich Repeats (LRRs). These occur in diverse proteins and function as sites of protein–protein interaction, peptide–ligand binding, and protein–carbohydrate interaction (reviewed Kobe and Deisenhofer, 1995; Jones and Jones, 1996). While the precise role of LRR domains in *R* gene function is unknown, Jonathan Jones (Sainsbury Lab, Norwich, United Kingdom) showed that the products of four cloned tomato *Cf* resistance genes are predicted to consist almost entirely of LRR units, with a putative membrane anchor and a small cytoplasmic domain at their C terminus. Nearly all amino acid differences between Cf proteins are in the amino-terminal 30% of the LRR domains, suggesting that they determine specificity. These amino-terminal LRRs are highly variable. In a core of nine amino acids, both synonymous and nonsynonymous base pair substitutions occur at frequencies similar to that observed in MHC peptide binding sites, and in ratios (compared to the rest of the protein) indicative of positive selection.

A second class of resistance proteins is defined by C-terminal LRR domains and three conserved motifs

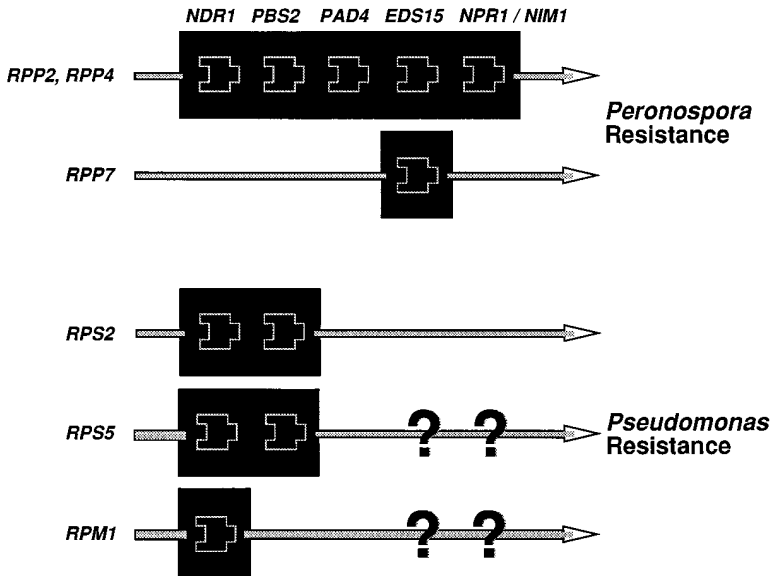

Figure 1. Genetic Analysis of Disease Resistance in Arabidopsis

A black box summary of signal transduction in the Arabidopsis accession Col-0, initiated by *R* genes that detect different isolates of eukaryotic and prokaryotic pathogens and culminate with effective disease resistance. The signaling genes (*NDR1*, *PBS2*, *PAD4*, *EDS15*, and *NPR1*) represent a small subset of genes that have been identified by mutation in Col-0 that are required for *R* gene function. Each of these mutants modifies the function of one or more *R* gene(s). Their relative position in the signaling process is currently unknown, and a question mark indicates interactions that have not yet been examined.

defining a nucleotide (ATP or GTP) binding site. This class of R proteins, christened "nucleotide-binding site plus leucine-rich repeat" or NB-LRR, is the largest to which specific resistance function can be ascribed. Both degenerate PCR experiments (Kanazin et al., 1996; Leister et al., 1996; Yu et al., 1996) and the Arabidopsis genome sequencing initiative (http://genome-www.stanford.edu/Arabidopsis/EST/R-EST_main.html) have uncovered a great number of these sequences, many of which map to regions harboring clusters of functionally defined resistance specificities (see Figure 1). The original members of NB-LRR class can be subdivided: *RPS2* and *RPM1* encode putative Leucine Zipper domains at their N termini, while *N*, *L6*, and *RPP5* encode a domain with homology to the intercellular signaling domains of the Drosophila TOLL and mammalian Interleukin 1 receptors, termed the TIR. Computer predictions offer no solid clue as to their subcellular localization.

Jeff Ellis (CSIRO, Canberra, Australia) described his group's dissection of the Flax *L* locus, one of the classic examples used to define the specific nature of plant-pathogen recognition several decades ago. Fourteen alleles have been described at *L*, one of which does not confer resistance to any known isolate of Flax rust. Following on the identification of *L6* as a TIR-NB protein with two directly repeated 150 amino acid domains, Ellis described the structure of five additional *L* alleles. They are similar, and most variability is in the leucine-rich regions. The number of leucine-rich domains can be more or less than two, and internal deletions are observed. Ellis used both constructed chimeric *L* alleles and naturally occurring intra-allelic recombinants to show convincingly that the leucine-rich domains are major determinants of specificity. In addition, he presented tantalizing data suggesting that while specificity is largely determined in the leucine-rich regions, intramolecular interaction with sequences in the TIR is required for full function.

Roger Innes (Indiana University, Bloomington, IN) described the *RPS5* LZ-NB-LRR gene in Arabidopsis encoding resistance to isolates of Pseudomonas syringae expressing the appropriate *avr* gene. Interestingly, an *rps5* mutant allele was selected that also dampens the function of different *R* specificities recognizing either P. syringae or Peronospora (with Eric Holub, HRI-Wellesbourne, UK). This allele carries a missense mutation that Innes postulates will be critical in intermolecular interactions, and he suggests that its negative effects on other *R* functions is via titration of a critical signal component.

Cell Biology and Localization of NB-LRR Proteins

The cellular localization of NB-LRR proteins and the role of their conserved structural domains in transducing the specificity of *avr* signals are still mysteries. A functional requirement for the NB was described by Barbara Baker (USDA Plant Gene Expression Center, Albany, California). Using the tobacco *N* gene, which encodes resistance against Tobacco Mosaic Virus, Dinesh Kumar in Baker's lab engineered P-loop mutations based on known Ras phenotypes. Some gave dominant-negative or weak allele phenotypes when transformed into *N* plants and were nonfunctional in *n* backgrounds. Many of the weak allele phenotypes are accompanied by a virus-induced, spreading hypersensitive cell death. This "rolling HR," as it has been dubbed by Chris Lamb, maintains the temperature sensitivity known to govern *N* function, lending credence to the notion that these are in fact weak alleles. The dominant-negative mutations raise the possibility that N functions as a dimer and/or that function or binding of auxiliary proteins are compromised by *N* mutations. TIR and LRR mutations also eliminated *N* function, demonstrating that the predicted structural domains are necessary for N function. Doug Boyes (Dangl lab, University of North Carolina, Chapel Hill, NC) presented immunoprecipitation data suggesting that the Arabidopsis LZ-NB-LRR protein RPM1 is peripherally membrane-associated. The fact that this first subcellular localization of an NB-LRR protein in a plant cell is noteworthy speaks to the amount of work still required.

The Kinase Connection

The Pto resistance protein of tomato is a cytoplasmic kinase defining an additional *R* gene class. It determines

resistance to P. syringae isolates expressing the corresponding *avrPto* gene. Recent observations suggest that Pto function is triggered by direct AvrPto–Pto interaction (Scofield et al., 1996; Tang et al., 1996), utilizes a phosphorylation cascade involving a second kinase, Pti1, which interacts with Pto (Zhou et al., 1995), and requires an LZ-NB-LRR protein called Prf (Salmeron et al., 1996). Greg Martin (Purdue University, West Lafayette, IN) reported that AvrPto mediates interaction of two Pto molecules in a yeast three-hybrid system. While Pto does not phosphorylate AvrPto, Martin proposes that the bacterial protein is the molecular bridge that affects cross-phosphorylation of Pto monomers. Martin's lab has narrowed down the region of Pto that confers recognition specificity for AvrPto to two amino acids predicted to be near a hydrophobic pocket, consistent with structural data from other Ser-Thr kinases. Finally, he discussed Pto interactors in addition to Pti1 (Zhou et al., 1997). These belong to a DNA binding protein family that includes factors implicated in regulating ethylene response genes (EREB proteins). Pti5 and Pti6 bind the same *cis* element as the EREBs, and these *cis* sequences exist in some plant defense gene promoters. In Martin's current model, Pti1 may be involved in regulating the HR, with Pti5 and Pti6 utilized to regulate at least part of the defense gene battery (see below). But this model still needs to account for the clear genetic requirement for *Prf*.

The final *R* gene structural class combines extracellular LRRs, like Cf proteins, with a Pto-like kinase domain. The prototype is Xa21, a rice receptor–like kinase determining resistance to Xanthomonas oryzae. There are seven *Xa21* gene family members at the locus from which the gene was cloned. Pam Ronald (University of California, Davis) described a role for transposon insertion and duplication as generators of duplication and diversity at *Xa21* (Song et al., 1997). *Xa21* is effective against 29/32 X. oryzae field isolates and transgenic *Xa21* expression in two high-yield rice cultivars planted on over 2.5 million hectares in Asia protects against infection. These two cultivars suffer serious losses due to X. oryzae infection, highlighting the agronomic relevance of this research. In an effort to recognize the importance of diverse germplasm sources (*Xa21* was originally introgressed from a wild rice relative from West Africa), Ronald has reached an agreement with University of California, Davis whereby a portion of future royalties arising from this and other research at University of California, Davis reliant on imported germplasm will support training of scientists from developing countries.

Other LRR-containing proteins play diverse roles in plant development and cell biology (Jones and Jones, 1996). Giulia DeLorenzo (University of Rome, Italy) summarized analyses of the polygalacturonase (PG) inhibitor proteins from tomato and bean (PGIPs; De Lorenzo et al., 1994). These are extracellular, consist entirely of LRRs, and bind to and inhibit fungal PG proteins. The latter are plant cell wall–degrading enzymes and potential pathogenicity factors. Four PGIP genes have been isolated from bean. Two have been expressed in heterologous systems, and characterization of the proteins shows that amino acid substitution is sufficient to confer changed recognition of PGs. Analysis of the structural basis of PGIP–PG interaction may provide a model for how plants' LRR proteins have evolved to recognize pathogen-derived molecules.

Evolution of Complex *R* Loci

As alluded to above, *R* genes are commonly organized as clusters in plant genomes. These clusters presumably provide a selective advantage by pyramiding numerous specificities that will protect a host individual against many parasite genotypes, and benefit the host species by providing a reservoir of genetic material from which new specificities can evolve. Jones compared DNA sequence and genome organization of the *Cf* homologs (*Hcf*) at the locus containing either *Cf-9*, *Cf-4*, or the allelic position in tomato cultivars lacking known *Cf* function. The intergenic regions of allelic positions within the *Hcr* loci are divergent in organization, but homologous in sequence. This may provide templates for unequal recombination and may also inhibit homogenization of the coding sequences.

Similar mechanisms probably exist at other complex loci. Scot Hulbert (Kansas State University, Manhattan, KS) described a cluster of Puccinia sorghi (rust) resistance genes in maize. From genetic evidence, the cluster contains at least three loci including the complex locus *Rp1*. This locus is a region of less than 1 cM that contains a variable number of *R* genes that recombine frequently, via both gene conversion and unequal crossing-over that can cause duplication and deletion of genes. New combinations of *R* genes from parental haplotypes as well as genes with altered phenotypes such as lesion mimics and novel resistance genes derived from two susceptible parents have been observed (Richter et al., 1995; Hu et al., 1996).

Evidence for unequal crossing-over driving the recent evolution of *R*-gene clusters has, however, not been found from extensive medium-range DNA sequencing at the *Pto* locus in tomato and in the major cluster of downy mildew resistance (*Dm*) genes in lettuce. Richard Michelmore (University of California, Davis) described a comparison among members of the *Pto* family from both resistant and susceptible haplotypes, and sequence comparisons between homologs of an NB-LRR gene cluster in cultivated and wild genotypes of lettuce. Sequence divergence among homologs within a given gene cluster appears to be ancient with no evidence of gene conversion events homogenizing family members. In addition, sequence comparisons of the NB regions from more than 50 NB-LRR gene fragments from lettuce (including a candidate *Dm3* gene) and other plant species indicated the presence of ancient families of NB-LRR genes that perhaps predate the divergence between monocot and dicot species.

The biotrophic oomycete Peronospora parasitica (downy mildew) is an excellent model eukaryotic parasite for genetic analysis of disease resistance in Arabidopsis, and more than twenty *RPP* specificities for downy mildew resistance have been identified genetically (reviewed by Holub and Beynon, 1996). Holub described how most of these reside in clusters, including two major *RPP* clusters on chromosomes 3 and 5 (*MRC-F* and *MRC-J*, respectively). *MRC-F* contains

RPP1, which is in the first complex locus (<1 cM region) identified in Arabidopsis shown by genetic recombination, mutation, and transformation experiments to contain several *R* specificities. Genes for resistance to other pathogens have been identified in *MRC-J* by several groups, including resistance to viruses, three different bacterial species, and the oomycete Albugo candida. Holub also described F2 segregation data from two self-fertile isolates of Peronospora including evidence for a single avirulence gene corresponding to *RPP1*, both confirming that the gene-for-gene relationship exists in this pathosystem and opening the doors of genetics in this parasite.

In this pathosystem, Jane Parker (Sainsbury Lab, Norwich, United Kingdom) described isolation of *RPP5*, encoding a TIR-NB-LRR protein (Parker et al., 1997). In Arabidopsis accession Ler-0, which expresses *RPP5*, there is only one functional copy and six partial homologs. In addition, there is an expressed open reading frame encoding a protein identical to the amino-terminal region of *RPP5* (termed homolog 1). This is a new twist on the alternative splice products encoding essentially the same domain observed by Ellis and Baker for *L6* and *N*, respectively. Baker presented data suggesting that both are required for correct *N* function, but the identification of *RPP5* via complementation did not include coexpression of the homolog 1. In the recipient accession for Parker's complementation test, Col-0, 7 of 8 *rpp* homologs are transcribable, but there is no obvious homolog 1 allele in the cluster. This suggests that homolog 1 is not required for *RPP5* function, or that a redundant function is present in Col-0.

Signals and Downstream Effectors of *R* Gene Action

Early signaling events following *R* gene engagement are calcium influx, K^+–H^+ exchange leading to alkalinization of the extracellular space, and an oxidative burst, all of which may have a direct role in halting further pathogen growth. These lead to transcriptional activation of a battery of defense-related genes in and surrounding the infected cell, resulting in salicylic acid (SA) biosynthesis, cell wall strengthening, lignification, production of various antimicrobial compounds, and the HR among others (reviewed by Dangl et al., 1996; Hammond-Kosack and Jones, 1996a). This local defense response stimulates establishment of pathogen-nonspecific systemic acquired resistance (SAR; reviewed by Ryals et al., 1996). SA accumulates to very high levels locally at infection sites undergoing HR. In at least tobacco and Arabidopsis, enzymatic blocking of salicylic acid (SA) accumulation subsequent to infection seriously impairs function of at least some *R* genes, and SA is required in distal tissues for SAR. It is unclear whether these signaling events are components of the same linear pathway or represent interdigitating signal pathways. Most importantly, it is unclear which of these events are causal mediators of *R* gene action, meaning that they lead directly to halting pathogen growth.

Loci Required for R *Gene Function and Pathogen Containment*

Genetic screens have identified loci required for *R* gene action, and it is likely that some encode proteins that mediate the series of events outlined above. Parker described a mutation, *eds1* for *e*nhanced *d*isease *s*usceptibility (Parker et al., 1996), that abolishes function of most known *RPP* specificities (except *RPP8*, which the Dangl lab has putatively identified as an LZ-NB-LRR) as well as P. syringae resistance conferred by *RPS4*. It also partially modifies "nonhost" resistance to isolates of Peronospora and Albugo candida (white blister) isolated from Brassica. In addition to its lack of effect on *RPP8*, *eds1* also does not appear to abolish function of other P. syringae *R* genes encoding LZ-NB-LRR domains, suggesting that it may be specifically required by TIR domain containing R proteins.

While most attention has focused on screens for loss of resistance, Fred Ausubel (Massachusetts General Hospital, Boston, MA) described mutants that express enhanced disease susceptibility following infection with subclinical doses of P. syringae (Glazebrook et al., 1997; Rogers and Ausubel, 1997). Twenty-seven *eds* mutants define at least 11 new loci required for limiting virulent pathogen growth. Some of these are impaired in SAR induction, including three new *npr1*/*nim1* alleles (described below). However, at least one *eds* mutant impaired in local containment of pathogen spread is still able to efficiently induce SAR. Innes described additional mutants (termed *pbs1–pbs3*) from their screen for impaired *RPS5* function, and Andrew Bent (University of Illinois, Champaign-Urbana, IL) described a mutant that expresses bacterial resistance without an HR. Holub described how many of these mutations also affect Peronospora resistance, demonstrating that a large number of genes can play a pathogen-nonspecific role in disease resistance. For example, in addition to alteration of bacterial resistance, at least 14 of the mutants available in the Col-0 impaired expression of Peronospora resistance conferred by *RPP4*.

These mutational analyses of disease resistance and pathogen containment in Arabidopsis are strengthened by the availability of a standard set of Pseudomonas and Peronospora isolates that trigger resistance through different *R* genes. Further characterization of these mutants will address a long-standing question, namely whether or not the role of *R* genes is to temporally hasten a basic pathogen-nonspecific response intrinsic to all plant cells. If so, then the prediction is that some *eds* loci will also turn out to be modifiers of *R* action. This prediction is partially born out by the finding that some *eds* isolates are alleles of the previously identified *pad* mutants, which can modify *RPP* gene function (Glazebrook et al., 1997).

Given the importance of SA in transducing pathogen signals, it is not surprising that several groups have sought mutants that are unable to establish SAR. Xinnian Dong (Duke University, Durham, NC) and Michelle Hunt (Novartis Crop Protection, Research Triangle Park, NC) described the independent isolations of the Arabidopsis *NPR1*/*NIM1* gene. Mutants in this gene lose the ability to respond to either pathogen or chemical signals that normally induce SAR. Additionally, they are compromised for at least some *R* functions and for the ability to limit the spread of a virulent pathogen (see above). The NPR1/NIM1 protein contains repeated ankyrin domains (Cao et al., 1997) that may have broad similarity

to IκB (Ryals et al., 1997). Dong provided evidence that overexpression of *NPR1* can lead to resistance against otherwise virulent pathogens. She also showed that an NPR1-GFP fusion protein complements the mutant phenotype and is localized to the nucleus upon activation of the SA-dependent resistance pathway by either SAR-inducing chemicals or avirulent pathogen.

Another class of mutants mimics either infection or the HR. Many of these mutants are constitutively "on" for conferring disease resistance to normally virulent pathogens when expressing lesions, arguing that they represent normal steps in the response to infection (reviewed by Briggs and Johal, 1994; Dangl et al., 1996). Jeff Dangl (University of North Carolina, Chapel Hill, NC) described the Arabidopsis *LSD1* gene, which encodes a new subclass of GATA-1-like zinc-finger protein (Dietrich et al., 1997). The *lsd1* mutant is normal under permissive conditions, but either pathogen or SAR-inducing chemicals applied locally can trigger local cell death and propagation of cell death throughout the leaf. Two functions for *LSD1* are inferred from the null phenotype: one is to dampen a pre-existing latent defense response, and the second is to control the extent of HR once it happens. Superoxide is a necessary and sufficient trigger of the null phenotype, suggesting that the wild type LSD1 protein interprets a superoxide-dependent signal from the oxidative burst to down-regulate the HR (Jabs et al., 1996). Dangl also described suppressors of the *lsd5* mutant that either modify *R* gene function or cross-suppress two other cell death mutants. These define loci coupling the cell death phenotypes with *R* function.

In addition to *LSD1*, the barley *MLO* gene also encodes a critical negative regulator of defense response. *mlo* mutants are resistant to all isolates of barley powdery mildew fungus, an important agronomic pathogen. Most *mlo* alleles are also lesion mimics. Paul Schulze-Lefert (Sainsbury Laboratory, Norwich, United Kingdom) described the technical tour-de-force of cloning *MLO* from the larger-than-human barley genome (Büschges et al., 1997). The low gene density around *MLO* was useful in identifying candidate ORFs and may be a generally applicable tool in gene isolation in large cereal genomes. The predicted Mlo protein has multiple transmembrane domains and is the first member of a large, plant-specific gene family. The mutants express a "hair trigger" for the formation of cell wall appositions, which physically block fungal penetration, and for onset of defense responses. Schulze-Lefert described both histological and gene expression experiments that show that the *mlo* mutant response is markedly quicker than wild type. There are many *MLO* homologs in barley and Arabidopsis. Whether these are also negative regulators of plant defense or are involved in other cellular processes is an interesting future question.

As described in the introduction, most plants are resistant to the majority of pathogens. These are traditionally called "nonhost interactions": no pathogen isolate infects any host cultivar. How pathogens can overcome this basic resistance is not understood, nor is it known how much overlap there might be between control of specific *R*-mediated resistance and nonhost resistance. The evolution of compatibility in the maize–C. carbonum interaction was described by Steve Briggs (Pioneer Hybrid Int. Inc., Johnston, IA). Some isolates of this fungus have gained the ability to produce a complex toxin, possibly via horizontal gene transfer. Some maize cultivars are susceptible to this toxin by virtue of lacking activity at a reductase locus. The HC toxin acts on histone deactylase, and Briggs provided evidence suggesting that it acts by inhibiting defense gene activation, which could normally require chromatin remodeling. Toxin-deficient fungal isolates cause an HR on all tested maize, defining this as a classic nonhost interaction. Briggs has now identified at least 42 independent mutants that exhibit partial susceptibility to these toxin minus, avirulent C. carbonum isolates, as measured by fungal colonization and host tissue destruction. These mutants define host components that may act beyond the coevolved *R* gene–dependent systems. It will be additionally important to address whether these mutants are also now generally susceptible to a variety of pathogens and whether they in fact modify known maize *R* genes.

Intracellular Response Regulators: Cell Biology

The relative lack of progress in understanding the biochemistry of signaling events leading to disease resistance stems in no small part from the lack of cultured cell systems that maintain specificity. This situation is changing for the better. Chris Lamb (Salk Institute, La Jolla, CA) and his colleagues analyze *R*-dependent events leading to cell death in cultured soybean cells (Levine et al., 1994, 1996; Shirasu et al., 1997). The order of events in this system is: avr-R engagement, calcium influx, reactive oxygen intermediate (ROI) production, larger calcium release, and apoptosis-like cell death morphologies. ROI act as early diffusable signals leading to activation of detoxification systems in surrounding cells. Salicylic acid potentiates these responses synergistically with bacteria expressing the appropriate *avr* gene. Lamb demonstrated that ROI accumulation at primary infection sites is necessary and sufficient for establishment of SAR in distal leaves. He further described "micro-HR" sites of 2–6 dead cells that appear in distal tissue after either inoculation of bacteria or exogenous generation of ROI in primary leaves. Approximately 2.5 times more micro-HRs appear after inoculation of avirulent bacteria than after isogenic virulent bacteria. When the oxidative burst is inhibited in primary infection sites, micro-HR formation in distal tissue is delayed. The formation of these micro-HRs near vascular bundles led Lamb to propose that the phloem mobile signal required for SAR signals is important for their establishment.

Detailed dissection of cell signaling events was provided by Dierk Scheel (IBP, Halle, Germany) using a purified peptide elicitor of nonhost defense from a fungal pathogen and parsley cell cultures (Nürnberger et al., 1994). Primary events of anion flux, oxidative burst, and target protein phosphorylation occur within the first few minutes. Inhibition of chloride channels blocks the other two signal events and subsequent defense gene activation (Jabs et al., 1997). Inhibition of membrane NADPH oxidase activity has no effect on channel activity but blocks oxidative burst and defense gene activation, thus setting a relative order for these events. Superoxide is the key ROI in this system, and Scheel's lab is taking the hard road to purify the membrane-bound NADPH oxidase complex. In addition, his lab has identified a

MAP kinase that is elicitor-activated downstream of anion channel function and either upstream or independent of ROI production.

Further validation of the use of cell cultures was described by Jones, using transgenic tobacco expressing the tomato *Cf-9* gene discussed above. Both the type and relative order of signal events following stimulation with the specific *avr9* peptide elicitor is consistent with that described above. His group has also identified a putative MAP kinase activity that is rapidly induced and dependent on calcium influx and protein phosphorylation. Like the parsley MAP kinase, this activity is either upstream or independent of ROI production.

Understanding Pathogenicity

Why do pathogens continue to express *avr* genes whose products limit the number of host genotypes they can successfully colonize? Quantitative losses of virulence have been observed for insertion mutants of several P. syringae *avr* genes, leading to suggestions that most pathogen isolates carry a battery of virulence factors, each contributing to virulence. Some of these also trigger *R* function. For example, Wolfgang Knogge (Max Planck Institute, Köln, Germany) described the *Nip1* gene in the imperfect fungus Rhynchosporium secalis (barley leaf scald) that encodes a host cell–toxic, cysteine-rich protein required for virulence, but that also acts as a genotype specific avirulence gene when cultivars carrying the *Rrs1* gene are inoculated with the fungus (Rohe et al., 1995). Cysteine-rich proteins were previously isolated as the avirulence gene products that correspond with Cf-4 and Cf-9 resistance in tomato (Van den Ackerveken et al., 1992; Joosten et al., 1994), as described by Pierre DeWit (Wageningen Agricultural University, Wageningen, The Netherlands). DeWit also presented evidence for other proteins that serve as virulence factors in the tomato-Cladosporium pathosystem (Laugé et al., 1997), including ECP2, which was said to elicit genotype-specific HR when injected into a collection of host accessions.

Plant bacterial pathogens require evolutionarily conserved Type III secretion systems to "deliver" virulence factors, analogous to systems used by bacterial pathogens of mammals (Alfano and Collmer, 1996). A major recent finding was that several P. syringae and X. campestris *avr* genes specifically trigger *R* function when expressed inside the plant cell (reviewed by Bonas and Van den Ackerveken, 1997). In this regard, Brian Staskawicz described a putative processing event for avrRpt2 that may occur inside the plant cell. He also presented evidence that large C-terminal epitope tags block delivery from P. syringae of both avirulence (when tested on Arabidopsis expressing the *RPS2* gene) and virulence (tested on an *rps2* mutant). Expression of the same tagged avrRpt2 constructs inside the plant cell still resulted in delivery of avirulence to *RPS2* plants. Smaller epitopes do not effect avrRpt2 delivery. This strongly suggests that the tagged protein is interfering with the Type III secretion system. Ulla Bonas described her group's finding that the Xanthomonas avrBs3 protein is functional inside pepper cells and is localized to the nucleus in onion cells (Van den Ackerveken et al., 1996). Mutants removing two of three NLSs in avrBs3 abolish both function and localization. An avrBs3 homolog, avrBs32, directs recognition of Xanthomonas inside cells of resistant tomato plants. In this case, however, the NLSs are not required, suggesting that recognition occurs in a nonnuclear compartment. These, and experiments like them in other labs, are crucial in defining all of the proteins that traverse the Type III injection system and in identifying their cellular targets in susceptible plants.

Several groups are using both genetics and medium-range sequencing to find more loci in the regulon controlled by the transcriptional activator of the Type III secretion system's genes. Matthieu Arlat (CNRS-INRA, Toulouse, France) identified several new ORFs in the bacterial wilt pathogen P. solanacearum (new name Ralstonia solanacearum). Deletion mutants of these often display attenuated virulence and weakened ability to trigger HR. One new ORF encodes a relative of TonB-dependent siderophore receptors, which they term *PrhA*. *PrhA* is not iron-regulated, but its deletion abolishes plant-dependent induction of the Type III transcription units. This is an exciting result, as it begins dissection of the processes that may control some of the earliest events in plant–pathogen interactions.

Mechanisms of fungal pathogenesis are also yielding to new tools of analysis. Regina Kahmann (University of München, Germany) described a GFP-based REMI screen in the corn smut pathogen Ustilago maydis for identification of plant-inducible fungal genes that may encode virulence factors. At least two new virulence genes have been isolated, a putative transcription factor and a seven-transmembrane protein. Using subtractive cloning and differential display to find genes regulated by the key U. maydis pathogenicity locus, *b*, they identified a cellulase gene (Schauwecker et al., 1995). This target of *b* regulation provides an easy screening tool for identification of mutants leading to constitutive cellulase activity. A gene encoding a protein with similarity to the large retinoblastoma (Rb) binding protein was thus identified that represses several but not all differentially expressed genes in the absence of *b* activity. A deletion mutant of this new gene is sporulation-defective but can still cause tumors in a *b*-activated background, thus separating these two important aspects of pathogenicity.

The variety of diseases caused by plant viruses is staggering, and an important current goal is to understand mechanisms of viral replication and systemic movement as potential points of interdiction in the viral life cycle. Jim Carrington (Washington State University, Pullman, WA) established a tobacco etch virus (TEV)-Arabidopsis pathosystem to identify the mechanisms of viral replication and movement. One viral protein, HC-pro (a proteolytic enzyme that cleaves its own precursor), is essential for sustained RNA replication and long-distance movement through the vasculature. This could occur via stimulation of necessary host factors or by suppression of host defense response. Long-distance TEV movement is restricted in Arabidopsis accessions, including Col-0, containing the dominant *RTM1* locus Col-0. Novel Col-0 mutants that allow viral movement were selected by infection with a recombinant TEV expressing a herbicide resistance gene. Mutants allowing

systemic viral spread were selected as BASTA-resistant. This will be a fascinating source of new loci required for inhibition of viral spread.

The interplay between virus resistance mechanisms and the phenomenon of gene silencing will yield new insights into how plant cells control transcriptional homeostasis. David Baulcombe (Sainsbury Lab, Norwich, United Kingdom) discussed both the interaction of potato virus X (PVX) with the *Rx* resistance gene in potato and PVX-induced gene silencing. His group has shown that PVX carrying sequences from a target gene will silence both the target gene and the viral genome if the nucleotide homology is not below 68% (Ratcliff et al., 1997). He drew parallels between PVX-induced silencing and the phenomenon of recovery seen when virus-infected plants lose symptoms and viral transcripts as they mature. PVX-induced silencing can be used to make mutant phenocopies of host genes in tobacco where the virus will spread systemically and silence the host gene in all cells (Angell and Baulcombe, 1997). Additionally, PVX induces silencing in transgenic Arabidopsis (which does not allow systemic spread of PVX) after transformation with an expressed copy of the viral genome including part of the open reading frame of the gene to be silenced. In two examples, all transgenic plants silenced the target gene. This "fast forward genetics" use of PVX-induced gene silencing, as coined by Baulcombe, will allow silencing of *Rx* to study its action and is sure to be an unparalleled tool in reverse genetics in both Solanaceous species and Arabidopsis.

This meeting's relaxing, isolated seaside setting focused attention on the key points in this exploding field. The first blush of cloning *R* genes is past, and the field is moving both up and down a beach littered with exciting discoveries still to be made. Continued concentration on highly developed genetic models like Arabidopsis, and exploitation of the genetic fruits of decades of plant breeding, will make genetics a key component of future advances. But the need for developments in cell biology and biochemistry will challenge current and future researchers in this area and will engage the participants at the next Italian feast in plant–microbe interactions. We thank Sarah Grant for contributions to the summary of some presentations and apologize to those participants whose work could not be discussed due to space limitations.

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